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NEWS	7	DEC 21	IPC search and display fields enhanced in CA/CAPLUS with the IPC reform
NEWS	8	DEC 23	New IPC8 SEARCH, DISPLAY, and SELECT fields in USPATFULL/USPAT2
NEWS	9	JAN 13	IPC 8 searching in IFIPAT, IFIUIDB, and IFICDB
NEWS	10	JAN 13	New IPC 8 SEARCH, DISPLAY, and SELECT enhancements added to INPADOC
NEWS	11	JAN 17	Pre-1988 INPI data added to MARPAT
NEWS	12	JAN 17	IPC 8 in the WPI family of databases including WPIFV
NEWS	13	JAN 30	Saved answer limit increased
NEWS	14	JAN 31	Monthly current-awareness alert (SDI) frequency added to TULSA
NEWS	15	FEB 21	STN AnaVist, Version 1.1, lets you share your STN AnaVist visualization results
NEWS	16	FEB 22	Status of current WO (PCT) information on STN
NEWS	17	FEB 22	The IPC thesaurus added to additional patent databases on STN
NEWS	18	FEB 22	Updates in EPFULL; IPC 8 enhancements added

NEWS EXPRESS FEBRUARY 15 CURRENT VERSION FOR WINDOWS IS V8.01a, CURRENT MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP), AND CURRENT DISCOVER FILE IS DATED 19 DECEMBER 2005. V8.0 AND V8.01 USERS CAN OBTAIN THE UPGRADE TO V8.01a AT <http://download.cas.org/express/v8.0-Discover/>

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NEWS INTER	General Internet Information
NEWS LOGIN	Welcome Banner and News Items
NEWS PHONE	Direct Dial and Telecommunication Network Access to STN
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* * * * * STN Columbus * * * * *

FILE 'HOME' ENTERED AT 15:22:01 ON 27 FEB 2006

=> file medline		
COST IN U.S. DOLLARS	SINCE FILE	TOTAL
	ENTRY	SESSION
FULL ESTIMATED COST	0.21	0.21

FILE 'MEDLINE' ENTERED AT 15:22:09 ON 27 FEB 2006

FILE LAST UPDATED: 23 FEB 2006 (20060223/UP). FILE COVERS 1950 TO DATE.

On December 11, 2005, the 2006 MeSH terms were loaded.

The MEDLINE reload for 2006 is now (26 Feb.) available. For details on the 2006 reload, enter HELP RLOAD at an arrow prompt (=>).
See also:

<http://www.nlm.nih.gov/mesh/>
http://www.nlm.nih.gov/pubs/techbull/nd04/nd04_mesh.html
http://www.nlm.nih.gov/pubs/techbull/nd05/nd05_med_data_changes.html
http://www.nlm.nih.gov/pubs/techbull/nd05/nd05_2006_MeSH.html

OLDMEDLINE is covered back to 1950.

MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the MeSH 2006 vocabulary.

This file contains CAS Registry Numbers for easy and accurate substance identification.

```
=> s hedgehog
      3649 HEDGEHOG
      852 HEDGEHOGS
L1      4017 HEDGEHOG
      (HEDGEHOG OR HEDGEHOGS)
```

```
=> s myocardial
      244527 MYOCARDIAL
      3 MYOCARDIALS
L2      244527 MYOCARDIAL
      (MYOCARDIAL OR MYOCARDIALS)
```

```
=> s 12 and 11
L3      12 L2 AND L1
```

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=> s 13 not py>2000
      2934825 PY>2000
      (PY>20009999)
L4      9 L3 NOT PY>2000
```

```
=> d ibib 1-9
```

```
L4  ANSWER 1 OF 9      MEDLINE on STN
ACCESSION NUMBER: 2000465512      MEDLINE
DOCUMENT NUMBER: PubMed ID: 11021439
TITLE: Cardiomyopathy in captive African hedgehogs
      (Atelerix albiventris).
AUTHOR: Raymond J T; Garner M M
CORPORATE SOURCE: Northwest ZooPath, Snohomish, WA 98296-4815, USA.
SOURCE: Journal of veterinary diagnostic investigation : official
      publication of the American Association of Veterinary
      Laboratory Diagnosticians, Inc, (2000 Sep) Vol. 12, No. 5,
      pp. 468-72.
      Journal code: 9011490. ISSN: 1040-6387.
PUB. COUNTRY: United States
DOCUMENT TYPE: (CASE REPORTS)
      Journal; Article; (JOURNAL ARTICLE)
```

LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200101
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20010125

L4 ANSWER 2 OF 9 MEDLINE on STN
ACCESSION NUMBER: 96426701 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8828980
TITLE: How often has Lp(a) evolved?
AUTHOR: Lawn R M
CORPORATE SOURCE: Falk Cardiovascular Research Center, Stanford University
School of Medicine, CA 94305-5246, USA.
SOURCE: Clinical genetics, (1996 Apr) Vol. 49, No. 4, pp. 167-74.
Ref: 61
Journal code: 0253664. ISSN: 0009-9163.
PUB. COUNTRY: Denmark
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199612
ENTRY DATE: Entered STN: 19970128
Last Updated on STN: 19970128
Entered Medline: 19961210

L4 ANSWER 3 OF 9 MEDLINE on STN
ACCESSION NUMBER: 94273536 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8004994
TITLE: Microcalorimetric study on myocardial metabolism
in a hibernator and two nonhibernators at 20 degrees C and
37 degrees C.
AUTHOR: Ikomi-Kumm J; Monti M; Hanson A; Johansson B W
CORPORATE SOURCE: Department of Internal Medicine, Lund University Hospital,
Malmo, Sweden.
SOURCE: Cryobiology, (1994 Apr) Vol. 31, No. 2, pp. 133-43.
Journal code: 0006252. ISSN: 0011-2240.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Space Life Sciences
ENTRY MONTH: 199407
ENTRY DATE: Entered STN: 19940729
Last Updated on STN: 19940729
Entered Medline: 19940715

L4 ANSWER 4 OF 9 MEDLINE on STN
ACCESSION NUMBER: 91138357 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2286096
TITLE: Mechanical restitution at different temperatures in
papillary muscles from rabbit, rat, and hedgehog.
AUTHOR: Liu B; Wohlfart B; Johansson B W
CORPORATE SOURCE: Department of Pharmacology, University of Lund, Sweden.
SOURCE: Cryobiology, (1990 Dec) Vol. 27, No. 6, pp. 596-604.
Journal code: 0006252. ISSN: 0011-2240.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199103
ENTRY DATE: Entered STN: 19910412
Last Updated on STN: 19910412
Entered Medline: 19910326

L4 ANSWER 5 OF 9 MEDLINE on STN
ACCESSION NUMBER: 91065005 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2249456
TITLE: Effects of low temperature on contraction in papillary
muscles from rabbit, rat, and hedgehog.
AUTHOR: Liu B; Wohlfart B; Johansson B W
CORPORATE SOURCE: Department of Pharmacology, University of Lund, Sweden.
SOURCE: Cryobiology, (1990 Oct) Vol. 27, No. 5, pp. 539-46.
Journal code: 0006252. ISSN: 0011-2240.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199101
ENTRY DATE: Entered STN: 19910308
Last Updated on STN: 19910308
Entered Medline: 19910115

L4 ANSWER 6 OF 9 MEDLINE on STN
ACCESSION NUMBER: 87029426 MEDLINE
DOCUMENT NUMBER: PubMed ID: 3769518
TITLE: Effects of induced hypothermia on organ blood flow in a
hibernator and a nonhibernator.
AUTHOR: Sjoquist P O; Duker G; Johansson B W
SOURCE: Cryobiology, (1986 Oct) Vol. 23, No. 5, pp. 440-6.
Journal code: 0006252. ISSN: 0011-2240.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198611
ENTRY DATE: Entered STN: 19900302
Last Updated on STN: 19900302
Entered Medline: 19861125

L4 ANSWER 7 OF 9 MEDLINE on STN
ACCESSION NUMBER: 86108432 MEDLINE
DOCUMENT NUMBER: PubMed ID: 4085517
TITLE: Ventricular repolarization and fibrillation threshold in
hibernating species.
AUTHOR: Johansson B W
SOURCE: European heart journal, (1985 Nov) Vol. 6 Suppl D, pp.
53-62.
Journal code: 8006263. ISSN: 0195-668X.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: (CASE REPORTS)
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198603
ENTRY DATE: Entered STN: 19900321
Last Updated on STN: 19900321
Entered Medline: 19860311

L4 ANSWER 8 OF 9 MEDLINE on STN
ACCESSION NUMBER: 85100400 MEDLINE
DOCUMENT NUMBER: PubMed ID: 6518802
TITLE: Cardiac responses in relation to heart size.
AUTHOR: Johansson B W
SOURCE: Cryobiology, (1984 Dec) Vol. 21, No. 6, pp. 627-36.
Journal code: 0006252. ISSN: 0011-2240.
Report No.: NASA-85100400.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

FILE SEGMENT: Priority Journals; Space Life Sciences
ENTRY MONTH: 198502
ENTRY DATE: Entered STN: 19900320
Last Updated on STN: 19900320
Entered Medline: 19850226

L4 ANSWER 9 OF 9 MEDLINE on STN
ACCESSION NUMBER: 62045823 MEDLINE
DOCUMENT NUMBER: PubMed ID: 13904450
TITLE: Myocardial lactate concentration in guinea-pigs,
normothermic and hypothermic, and hedgehogs, in a
hibernating and a non-hibernating state.
AUTHOR: HANSON A; JOHANSSON B W
SOURCE: Acta physiologica Scandinavica, (1961 Oct) Vol. 53, pp.
137-41.
Journal code: 0370362. ISSN: 0001-6772.
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: OLDMEDLINE; NONMEDLINE
ENTRY MONTH: 199811
ENTRY DATE: Entered STN: 19990716
Last Updated on STN: 19990716
Entered Medline: 19981101

=> file pctfull

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	2.31	2.52

FILE 'PCTFULL' ENTERED AT 15:23:24 ON 27 FEB 2006
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FILE LAST UPDATED: 21 FEB 2006 <20060221/UPTX>
MOST RECENT UPDATE WEEK: 200607
FILE COVERS 1978 TO DATE

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FORMAT CHANGES <<<

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ONLY, USE FIELD CODE FPI <<<

>>> SDI SEARCHES (ALERTS) WILL BE RESUMED WHEN BIBLIOGRAPHIC DATA
BECOME AVAILABLE <<<

=> s hedgehog

1002 HEDGEHOG
55 HEDGEHOGS
L5 1029 HEDGEHOG
(HEDGEHOG OR HEDGEHOGS)

=> s myocardial

L6 17861 MYOCARDIAL

=> s 16 and 15

L7 165 L6 AND L5

=> s structure or formula or compound

418360 STRUCTURE
 206597 STRUCTURES
 455983 STRUCTURE
 (STRUCTURE OR STRUCTURES)
 151185 FORMULA
 24694 FORMULAS
 25119 FORMULAE
 158696 FORMULA
 (FORMULA OR FORMULAS OR FORMULAE)
 204205 COMPOUND
 215366 COMPOUNDS
 263248 COMPOUND
 (COMPOUND OR COMPOUNDS)

L8 578073 STRUCTURE OR FORMULA OR COMPOUND

=> s 18 and 17

L9 163 L8 AND L7

=> s 19 not py>1999

630082 PY>1999

L10 18 L9 NOT PY>1999

=> s agonist

25066 AGONIST

27468 AGONISTS

L11 34707 AGONIST

(AGONIST OR AGONISTS)

=> s l11 and l10

L12 6 L11 AND L10

=> d ibib 1-6

L12 ANSWER 1 OF 6 PCTFULL COPYRIGHT 2006 Univentio on STN

ACCESSION NUMBER: 2006009836 PCTFULL

no bibliographic data available - please use FPI for PI information

DESIGNATED STATES

L12 ANSWER 2 OF 6 PCTFULL COPYRIGHT 2006 Univentio on STN

ACCESSION NUMBER: 2006008342 PCTFULL

no bibliographic data available - please use FPI for PI information

DESIGNATED STATES

L12 ANSWER 3 OF 6 PCTFULL COPYRIGHT 2006 Univentio on STN

ACCESSION NUMBER: 2006006948 PCTFULL

no bibliographic data available - please use FPI for PI information

DESIGNATED STATES

L12 ANSWER 4 OF 6 PCTFULL COPYRIGHT 2006 Univentio on STN

ACCESSION NUMBER: 1999064627 PCTFULL ED 20020515

TITLE (ENGLISH): PROBES USED FOR GENETIC FILING

TITLE (FRENCH): SONDAS UTILISEES POUR PROFILAGE GENETIQUE

INVENTOR(S): ROBERTS, Gareth, Wyn

PATENT ASSIGNEE(S): GENOSTIC PHARMA LIMITED;

ROBERTS, Gareth, Wyn

LANGUAGE OF PUBL.: English

DOCUMENT TYPE: Patent

PATENT INFORMATION:

NUMBER	KIND	DATE
WO 9964627	A2	19991216

DESIGNATED STATES

W:

AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK
 EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP
 KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL

PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN
YU ZA ZW GH GM KE LS MW SD SL SZ UG ZW AM AZ BY KG KZ
MD RU TJ TM AT BE CH CY DE DK ES FI FR GB GR IE IT LU
MC NL PT SE BF BJ CF CG CI CM GA GN GW ML MR NE SN TD
TG

APPLICATION INFO.:

PRIORITY INFO.:

WO 1999-GB1780 A 19990604
GB 1998-9812099.1 19980606
GB 1998-9813291.3 19980620
GB 1998-9813611.2 19980624
GB 1998-9813835.7 19980627
GB 1998-9814110.4 19980701
GB 1998-9814580.8 19980707
GB 1998-9815438.8 19980716
GB 1998-9815576.5 19980718
GB 1998-9815574.0 19980718
GB 1998-9816085.6 19980724
GB 1998-9816086.4 19980724
GB 1998-9816921.2 19980805
GB 1998-9817097.0 19980807
GB 1998-9817200.0 19980808
GB 1998-9817632.4 19980814
GB 1998-9817943.5 19980819

L12 ANSWER 5 OF 6

ACCESSION NUMBER:

TITLE (ENGLISH):

TITLE (FRENCH):

INVENTOR(S):

PATENT ASSIGNEE(S):

LANGUAGE OF PUBL.:

DOCUMENT TYPE:

PATENT INFORMATION:

PCTFULL COPYRIGHT 2006 Univentio on STN
1999056785 PCTFULL ED 20020515
MUSCLE-DERIVED CELL MEDIATED GENE DELIVERY FOR TREATING
MUSCLE- AND BONE-RELATED INJURY OR DYSFUNCTION
TRANSPORT DE GENE EFFECTUE PAR L'INTERMEDIAIRE D'UNE
CELLULE DE MUSCLE PERMETTANT DE TRAITER LES LESIONS OU
LES DYSFONCTIONS MUSCULAIRES OU OSSEUSES
CHANCELLOR, Michael, B.;
HUARD, Johnny
UNIVERSITY OF PITTSBURGH
English
Patent

NUMBER	KIND	DATE
WO 9956785	A2	19991111

DESIGNATED STATES

W:

AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK
EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP
KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL
PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG UZ VN YU
ZA ZW GH GM KE LS MW SD SL SZ UG ZW AM AZ BY KG KZ MD
RU TJ TM AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC
NL PT SE BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG

APPLICATION INFO.:

PRIORITY INFO.:

WO 1999-US9451 A 19990430
US 1998-60/083,917 19980501

L12 ANSWER 6 OF 6

ACCESSION NUMBER:

TITLE (ENGLISH):

TITLE (FRENCH):

INVENTOR(S):

PATENT ASSIGNEE(S):

LANGUAGE OF PUBL.:

DOCUMENT TYPE:

PATENT INFORMATION:

PCTFULL COPYRIGHT 2006 Univentio on STN
1998035020 PCTFULL ED 20020514
METHODS FOR MODULATING HEMATOPOIESIS AND VASCULAR
GROWTH
PROCEDES DESTINES A MODULER L'HEMATOPOIESE ET LA
CROISSANCE VASCULAIRE
BARON, Margaret, H.;
FARRINGTON, Sarah, M.;
BELAOUSSOFF, Maria
THE PRESIDENTS AND FELLOWS OF HARVARD COLLEGE
English
Patent

NUMBER	KIND	DATE
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	WO 9835020	A2 19980813
DESIGNATED STATES		
W:	CA JP AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE	
APPLICATION INFO.:	WO 1998-US2633	A 19980210
PRIORITY INFO.:	US 1997-60/037,513	19970210
	US 1997-60/049,763	19970616

=> d kwic 6

L12 ANSWER 6 OF 6 PCTFULL COPYRIGHT 2006 Univentio on STN
 ABEN Methods and assays are provided for selecting compounds that are functionally equivalent to a gene product expressed in an embryo's extraembryonic tissue for use in modulating hematopoiesis and vascular growth, such compound being exemplified by a hedgehog protein, and an agonist of a hedgehog protein binding receptor. According to the method, such compound causes undifferentiated mesodermally derived cells to undergo at least one of hematopoiesis or vasculogenesis. Examples of undifferentiated mesodermally derived cells. . .

ABFR . . . d'un embryon. Ces procedes sont destines a moduler l'hematopoiese et la croissance vasculaire, le compose etant notamment une proteine a structure dite en herisson, ainsi qu'un agoniste d'un recepteur de liaison de proteine a structure dite en herisson. Conformement a ce procede, un tel compose permet de soumettre a hematopoiese ou developpement du systeme vasculaire. .

DETD . . . mesodermally derived cells, to undergo at least one of hematopoiesis and vascular growth. The method includes the steps of selecting a compound that is functionally equivalent to a gene product expressed in an embryo's extraembryonic tissue; and causing the compound to access the cells, so as to stimulate the cells to undergo at least one of hematopoiesis and vascular growth.

. . . in vascular growth or hematopoiesis in an embryo in utero, that includes the steps of: selecting an effective dose of a compound that is functionally equivalent to a gene product expressed in an extraembryonic tissue; and causing the compound to access a population of embryonic cells in vivo, so as to stimulate the cells to undergo at least one of hematopoiesis. . .

. . . treating a subject suffering from an abnormal number of erythroid cells, that includes the steps of selecting an effective dose of a compound that is functionally equivalent to a gene product expressed in an extraembryonic tissue; and causing the compound to access a population of hematopoietic stem cells over an effective time so as to modulate the number of cells undergoing. . .

. . . for treating a subject suffering from an ischemia in tissues containing mesodermally derived cells, that includes

selecting an effective dose of a compound that is functionally equivalent to a gene product expressed in an extraembryonic tissue; and administering the compound to the ischemic site over an effective time so as to stimulate vascular growth.

In another embodiment of the invention, an in vitro assay is provided for determining the activity of a compound capable of modulating hematopoiesis or vascular growth, that includes the steps of selecting a population of cells from a tissue derived.

In another embodiment of the invention, an assay is provided for determining the activity of a compound capable of modulating hematopoiesis or vascular growth, that includes the steps of selecting a first transgenic animal carrying a marker:E-globin hybrid
1. . . . an embryo from the mating at a time within the first third of the gestation period; and determining the effect of the compound on the stimulation of hematopoiesis and vascular growth in the isolated embryo by measuring marker expression.

Fig. 3 shows the formation of yolk sac-like structures by cultured blastocysts (a) transgenic blastocysts prior to culture (b) Sac-like structure (non transgenic) stained with benzidine to reveal hemoglobin containing cells (c) Sac from cultured transgenic blastocysts stained with XGal to reveal hemoglobin.

Fig. 4 shows RT-PCR analysis of blastocyst cultures: (A) e-globin was observed in blastocysts that have developed into sac-like structures (sac) but not in samples that were relatively flat mounds of cells (flat). The higher molecular weight band is the internal control-actin.. . .

but is absent in epiblasts only, as determined by XGal staining. Dashed lines were drawn around the epiblasts to facilitate visualization of structures. (a) whole embryo on a filter; (b) epiblast on a filter; (c) whole embryo on a slide; and (d) epiblast on.

Fig. 9 shows that recombinant hedgehog protein can substitute for visceral endoderm to stimulate primitive hematopoiesis in cultured epiblasts. Isolated epiblasts were cultured in the absence (lanes labeled none) or presence of three different concentrations of recombinant hedgehog protein (0.25, 1 and 5 Vg/ml). Primitive hematopoiesis was assessed by RT-PCR analysis for e-globin expression. Actin served as an internal.

The circular structure represents a blastocyst of around 3.5 days.

stem cells and progenitor cells from embryo or adult. Embodiments of the invention are further directed to novel assays for identifying

compounds capable of stimulating hematopoiesis and vascular growth. Support for the methods of the invention are provided in the examples contained herein. According to an embodiment of the invention,

compounds have been identified that are capable of stimulating blood development in the embryo and in the adult and are functionally equivalent to gene products expressed in the visceral endoderm and yolk sac mesoderm. Such gene products are exemplified by hedgehog

compounds, TGF- β , TNF, and WNT compounds and are here identified as achieving a similar effect to that observed with extraembryonic tissues with regard to hematopoiesis and vascular growth in undifferentiated mesodermal derived tissues. In an embodiment of the invention, compounds including those selected from hedgehog and TGF- β may act synergistically so as to enhance their stimulatory effect on target cells.

Synergistic effect is defined here as for two or more compounds where little or no biological effect is observed with the compounds alone but together the compounds have a potent biological effect.

Hedgehog compound is defined here and in the claims as a class of molecules of the

hedgehog family that includes recombinant hedgehog protein, analogs, and derivatives of

hedgehog proteins, and agonists and antagonists of hedgehog protein receptors and functional equivalents of the aforementioned.

and

in the adult. According to embodiments of the invention, processes of vascular growth and hematopoiesis in embryonic development are affected by compounds in the visceral endoderm. For example, we have identified for the first time that hedgehog proteins act on undifferentiated mesodermal derived cells in vitro to stimulate blood formation and on embryonic tissue and yolk sac development at very early stages in the hematopoiesis and vascular growth pathways. Furthermore, according to the invention, these early acting

compounds have utility in regulating hematopoiesis and vascular growth in the adult animal.

addition of visceral endoderm which is sufficient to cause the anterior epiblast to

form blood islands. When either visceral endoderm or hedgehog protein was added to the

culture, blood formation was observed. (Figure 16)

(iv) Explants or embryoid bodies derived from mutants defective in.

visceral endoderm such that its absence results in the failure to make blood, is a

suitable model system for screening novel compounds from libraries such as those derived

from extraembryonic tissues, where these libraries include combinatorial

peptide libraries and recombinant DNA libraries. By using a pooling strategy to reduce the number of experimental tests, compounds may be identified that are useful in modulating hematopoiesis and vascular growth in embryoid bodies.

type of assay can be used to study the effect of other mutations, such as deficiency of signaling factors such as hedgehog proteins (for example, Indian hedgehog), on blood formation. (Examples 3-5) For example, Ihh null mutant ES cells may be formed and factors capable of overcoming the mutation, identified.. These cells could be rescued either by providing exogenous hedgehog protein or by transfecting the cells with vectors expressing a hedgehog gene utilizing standard vectors or retroviral vectors. (Figure 9) The mutated cells could also be reintroduced into mice to form chimeras.

assay for expression of many genes from a single culture product. (Figure 4) Using the above assays, we have identified a number of compounds that are functionally equivalent to gene products that are expressed in extraembryonic tissues and may stimulate blood formation. These compounds include TGF- β proteins more specifically TGF- β I more specifically bone morphogenic protein (BMP) more specifically BMP-4; tumor necrosis factor (TNF) proteins more specifically TNF- α ; wnt family; and hedgehog proteins.

(Figures 5, 9 and 17) Compounds may also include naturally occurring and synthetic agonists, antagonists, analogs and derivatives of the above. These molecules may interact with membrane proteins which initiate signal transduction pathways resulting in a biological response. Therefore, in addition to the above compounds, agonists and antagonists to these membrane binding proteins including those receptors, receptor agonists and receptor antagonists associated with hedgehog binding receptors and hedgehog signalling transduction pathways such as smoothened, patched and gli may have utility in regulating hematopoiesis and vascular growth.

G) screening libraries of compounds for activity in stimulating hematopoiesis and vascular growth;
(ii) testing for the effect of growth factors, cytokines and other signaling molecules on embryonic hematopoiesis and also on vascular growth;
(iii) determining the effect of hedgehog proteins on hematopoiesis and vascular growth in the embryo, fetus and adult. For example, the blastocyst assay may be used to determine the effect of hedgehog proteins on yolk sac' development ex vivo where the blastocyst is derived from transgenic or non-transgenic animals.

mesoderm is of the same

origin as that of the yolk sac;
 (v) following the development of primitive erythroid cells and vascular structures by staining with a marker such as XGal so as to outline the vasculature and permit the tracking of vascular growth as. . . individual explants of targeted mutations in genes that affect hematopoiesis or vascular growth in the parent animal including those carrying transgenes expressing hedgehog, patched, Gli and other proteins; and
 (vii) examining the effect of gene therapy on mesodermally derived tissues; where for example, the gene for hedgehog protein is introduced into prestreak embryos deprived of the visceral endoderm, under various promoters so as to modulate the effect of. . .

Hedgehog proteins: We have shown here for the first time that hedgehog proteins are capable of stimulating hematopoiesis in the yolk sac, and the splanchnopleura and other hematopoietic tissues of the embryo or fetus. . . of the adult. (Examples 3-5, Tables 1-2, Figs 6,9). By screening for molecules that were present in the visceral endoderm, we identified hedgehog gene product. When a hedgehog protein (SHH) was added to epiblast cultures and RNA was isolated after 2-3 days and analyzed by RT-PCR (Example 3, Fig. . . .

The above assays show that hedgehog proteins expressed in extraembryonic tissue as well as hedgehog proteins that are closely related to proteins expressed in extraembryonic tissues, stimulate hematopoiesis and vasculogenesis. Members of the hedgehog family which are a distinct family of signaling molecules (e.g., reviewed in Goodrich et al., Genes & Develop. 10 (1996), 301-12) are known. . . spermatogenesis. The family was initially identified as involved in normal segmental patterning in Drosophila (Nusslein-Volhard et al, Nature, 287 (1980), 795-801). The hedgehog family includes Desert hedgehog (DHH) protein, Indian hedgehog protein (IHH), Moonratt hedgehog (Zebrafish) and Tiggly winkle hedgehog (Zebrafish).

The utility of the hedgehog proteins in stimulating hematopoiesis and vascular growth is further reinforced by our experiments on target molecules through which these proteins act.

In support of our observations that hedgehog proteins are capable of stimulating hematopoiesis, we identified the enriched expression of Gli and patched in yolk sac mesoderm. Gli is a transcription factor involved in the transduction pathway on which hedgehog proteins act, while PTC (patched) is a membrane protein that binds hedgehog protein to initiate the signal transduction pathway that ultimately causes a biological response in the target cell. The association of these proteins with yolk sac

mesoderm further supports the observation that hedgehog proteins stimulate hematopoiesis. Since ptc is the presumed gateway to a cell response, any agonist of hedgehog capable of binding patch is expected to induce the same biological effect as hedgehog-in this case, hematopoiesis and vascular growth.

Certain hedgehog proteins have been reported to be involved in the initiation of expression of the secondary signaling molecules-BMP-2 and BMP-4 (proteins belonging. . . to the TGF-P family) in the mesoderm and Fgf-4 in the ectoderm (WO 95/18856). We have identified for the first time, that hedgehog proteins might interact in a synergistic manner with secondary signaling molecules to stimulate hematopoiesis and vascular growth (Example 6).

The activity of compounds that are functional equivalents to a gene product expressed in extra-embryonic tissue such as recombinant hedgehog protein, analogs, derivatives and dissociation products of hedgehog proteins, and agonists of hedgehog protein receptors such as PTC according to the invention, may stimulate hematopoiesis and vascular growth by 1 5 acting on cells or. . . .

The invention includes the use of functional peptides of hedgehog protein. The term functional peptide as a subclass of a hedgehog compound defined above, is meant to include peptide fragments of the hedgehog protein that are capable of inducing a biological activity that is the same or equivalent to the entire protein (WO 96/16668, incorporated here by reference). The invention further includes hedgehog compounds described in WO 95/18856 and here incorporated by reference, including homologs of hedgehog proteins, recombinant hedgehog proteins, hedgehog encoding nucleic acids, antisense molecules, gene constructs for use in gene therapy including viral vectors known in the art, combinatorial mutants of hedgehog proteins as agonists or antagonists, and antibodies specific for hedgehog protein epitope. These and other compounds may be selected for modulating hematopoiesis and vascular growth according to the assays of the invention.

invention, these factors may be used to stimulate hematopoiesis and vascular growth in animals including mammals, including humans. Similarly antagonists to the compounds of the invention may be used to inhibit vascular growth and hematopoiesis.

Our novel blastocyst assay may be used to determine the effect of hedgehog proteins on yolk sac development. In addition, blastosacs could be assayed for gene expression not only using LacZ as a histochemical marker,

Transgenic mouse models for studying the effect of selected

compounds on hematopoiesis
and vascular growth.

al. J.Biol. Chem. Vol 270, (1995) pp 1289-1294). Other transgenic mice may be formed in which a selected sequence from the hedgehog gene family may be placed under control of an enhancer and/or promoter of the sort described above. Furthermore, transgenic mice may be generated in which the hedgehog or hedgehog agonist or antagonist is expressed under the control of heterologous tissue specific promoters/enhancers such as described above. Other transgenic animals may be formed in which hedgehog regulatory sequences are used to drive expression of heterologous gene coding sequences in specific embryonic or adult tissues eg Ihh regulatory sequences. . . .

Science vol 269 (1995)pp 679-682, to target hedgehog genes into selected sites in the genome under the control of endogenous sequences in embryonic stem (ES) cells. These modified ES cells. . . .

to blood diseases such as leukemias, and abnormal vascular growth and abnormal hematopoiesis. These events may be analyzed with regard to hedgehog compounds.

There are a number of therapeutic applications for compounds of the invention. Such uses are associated with the modulation of hematopoiesis and vascular growth and include methods that result in stimulation as well as those that result in inhibition of proliferation and/or differentiation of stem cells. Examples of compounds of the invention have been discussed above.

(a) therapeutic compounds such as hedgehog proteins including derivatives, analogs, and degradation products of naturally occurring proteins; agonists or antagonists of protein receptors as well as functional equivalents of the above listed compounds. The therapeutic compounds may be isolated from cultures of extra-embryonic tissues, manufactured by recombinant technology or prepared by synthetic chemistry;
(b) coding sequences for the above- listed therapeutic compounds, incorporated into vectors suited for gene therapy techniques; and
(C) mammalian cells that have been transformed with coding sequences of the above for. . . .

of the techniques available in the art. For example, a protein, analogue, derivative, antagonist or receptor, of an identified protein (collectively called compounds) such as hedgehog related compounds, may be introduced into a vector and the vector introduced into the appropriate target tissue where this tissue is located in an. . . . enhancer to ensure selective

expression in the targeted tissue. For example, use of the cardiac actin enhancer to express the desired compound in the heart, the MCK enhancer to express the compound in skeletal muscle; sca-I regulatory sequences to 1.5 express hedgehog compound in hematopoietic stem cells or a retina-specific regulatory element of the interphotoreceptor retinoid-binding protein to express the compound in the retina.

heterologous cells contained within an immune protective barrier, may be manipulated by standard techniques to secrete the selected protein such as hedgehog, or analogues, derivatives, antagonists or receptors of protein.

lineages. Examples of targets for such treatments include in vivo or in vitro exposure of undifferentiated mesodermally derived cells to a compound of the invention. Examples of target cells include bone marrow stem cells, progenitor cells, and cord blood cells. These cells may be . . . or the cells may be freshly isolated and maintained in vitro in a culture medium. Exposure of such cells to the compound results in enhanced proliferation and/or differentiation of the cells, the stimulated cells being implanted in the same or different subject from which. . . .

from disease caused by infectious agents such as human immune deficiency virus and may be treated using a method 1.0 and compounds that stimulate hematopoiesis. The consequences of such abnormalities if untreated are various forms of anemia (associated with abnormally low levels of erythrocytes)

degenerative disease, aging, trauma, or infectious agents. Examples include diabetic chronic ulcers, burns, frost bite, ischemic events following stroke and transplantation. The compounds of the invention may be used in the adult for induction of revascularization or formation of collateral vessels in ischemic myocardium or ischemic limbs, and in coronary artery bypasses and in promoting wound healing in general. For example, compounds of the invention may be used in treatment of duodenal ulcers by enhancing microvessel density and promoting more rapid healing. In. . .

5'-ACACGATGCCATGCTGGTCA-3'
c-mysin(5') 5'-CTCGCAGAACAGCAGCCTAA-3' PCR product is 679bp; 32 cycles
c-mysin(3') 5'-AGGGTCTGCTGGAGAGGTTA-3'
(C) BLASTOCYSTS ISOLATED AT ABOUT 3.5 DPC PROVIDE A
MODEL SYSTEM FOR SCREENING COMPOUNDS THAT CAN
STIMULATE HEMATOPOIESIS AND VASCULAR GROWTH OF
UNDIFFERENTIATED MESODERMAL CELLS
Blastocyst cultures were prepared and used to analyze the effects of
compounds on
the stimulation of undifferentiated mesodermal derived cells to undergo
hematopoiesis and
vasculogenesis. The blastocyst culture system described here is suited

for following the development of embryonic structures in vitro, such as the yolk sac, that normally form post implantation in vivo. The effects of exogenously added growth factors.

(2,000 U/ml), streptomycin (2,000 pg/ml), 2 mM glutamine, 1 mM pyruvate, 0.1 mM nonessential amino acids (GIBCO-BRL), and 10⁻⁴M β-mercaptoethanol. Sac-like structures could first be seen around 7 days in culture; by 9-10 days they had enlarged to the point where they were easily visible with the naked eye (0.2 mm in diameter). These sac-like structures (here termed blastosacs) closely resembled early in utero yolk sacs.

4A, embryonic globin is produced only when yolk sac-like structures form, but not if the blastocysts do not progress in their development beyond an amorphous mound of trophoblast cells.

Null mutant embryoid bodies. Embryoid bodies are structures derived from ES cells that form blood islands under appropriate culture conditions (Keller (1995)). We have developed an assay system using embryoid bodies. . . . 195) Gene Targeting: A Practical Approach (New York: IRL Press). with mutations in selected genes were rescued by addition of a compound that is functionally equivalent to the gene product expressed by the non-mutated gene.

Example 3: Compounds that are functionally equivalent to a gene product expressed in an embryo's extraembryonic tissue (exemplified by hedgehog protein) stimulate hematopoiesis and vascular growth of undifferentiated mesodermal cells (exemplified by epiblast mesoderm)

(a) A hedgehog protein, typified by Sonic hedgehog, was demonstrated to stimulate hematopoiesis in the epiblast mesoderm using the method of Example 2(A) (Fig. 9).

(b) Compounds that are functionally equivalent to a gene product expressed in an embryo's extraembryonic tissue (exemplified by hedgehog protein) stimulate hematopoiesis and vascular growth of undifferentiated mesodermal cells (exemplified by adult bone marrow cells).

To determine whether recombinant hedgehog proteins influence the development or differentiation of adult hematopoietic stem or progenitor cells, we carried out in vitro clonal assays. Mononuclear cells. . . .

bovine serum albumin (cell culture grade BSA, 1%), 2-mercaptoethanol (1 x 10⁻⁶M) and the indicated growth factors and recombinant hedgehog proteins. Recombinant human erythropoietin (Epo) was obtained from Amgen and used at 40

U/nil. Recombinant interleukin-3 (IL-3) and granulocyte/macrophage-colony stimulating factor (GM-CSF) were. . . were scored on the days indicted. Colonies were scored as CFU-E, BFU-E, myeloid or mixed. Where included in the cultures, recombinant hedgehog proteins were added at concentrations between 1 and 5 yg/ml. Buffer alone (5 mM sodium phosphate pH 5.5 150mM NaCl, 0.5 mM. . . .

all types (erythroid: CFU-E, BFU-E; myeloid: CFU-GM) were increased by - 1.5 to more than 4-fold, in a dose-dependent manner (recombinant hedgehog protein added at 1, 2.5, 5yg/ml, X ug). The observation that hedgehog proteins are apparently not selective for erythroid versus myeloid lineage is consistent with the hypothesis that they stimulate stem or early. . . .

All three recombinant hedgehog proteins stimulated colony formation. From these data we conclude that both SHH and IHH enhance proliferation, differentiation and/or survival of hematopoietic stem/progenitor. . . .

were stored in buffer pH 8.0; untagged SHH was stored in buffer pH 5

Other approaches to measuring the effect of compounds that are functionally equivalent to a gene product expressed in an embryo's extraembryonic tissue on undifferentiated mesodermal cells.

by flow cytometry (fluorescence-activated cell sorting, FACS) or magnetic immunoselection (Testa and Molineux, 1993) and their development enhanced in the presence of hedgehog protein. These resulting populations are examined using in vivo assays include the CFU-S assay (spleen colony-forming unit) and long-term bone marrow cultures. . . .

sac mesoderm. (Fig. 6) The enriched expression of Gli and patched in yolk sac mesoderm points to mesoderm as target of hedgehog signalling.: Yolk sacs from 10.5 and 12.5 dpc embryos were separated into endoderm (e) and mesoderm (m) fractions and RNA was prepared. . . .

Example 6: Synergistic effect of Hedgehog protein with TGF- β proteins on

1.5 hematopoiesis (and vascular growth)

Using the methods of Example 3(A) above, we have shown using RT-PCR, that both

Indian Hedgehog and BMP-6 are expressed in early visceral endoderm. Whole embryo

(6.5dpc), epiblasts, epiblasts plus hedgehog protein,

epiblasts plus BMP-6 protein and

epiblasts plus hedgehog protein and BMP-6; are examined after 72 hrs incubation to

determine the extent of activation of E-globin expression. The experiment is repeated for

BMP-2, BMP-4 and BMP-6. We expect to observe an enhanced effect when both hedgehog

and BMP-4 are present compared with either alone.

CLMEN. . . stimulating a population of undifferentiated mesodermally derived cells to undergo at least one of hematopoiesis and vascular growth; comprising:
(a) selecting a compound that is functionally equivalent to a gene product expressed in an embryo's extraembryonic tissue;
(b) causing the compound to access the cells, so as to stimulate the cells to undergo at least one hematopoiesis and vascular growth.

2 A method according to claim 1, wherein the compound is a secreted protein.

3 A method according to claim 1, wherein the compound is a hedgehog compound.

4 A method according to claim 3, wherein the compound is an agonist of a hedgehog protein binding receptor.

5 A method according to claim 4, wherein the hedgehog protein binding receptor is patched.

6 A method according to claim 1, wherein the compound causes enriched expression of Gli.

7 A method according to claim 3, wherein the hedgehog compound is selected from the group consisting of Indian hedgehog, Desert hedgehog and Sonic hedgehog compound.

8 A method according to claim 3, wherein the compound is an Indian hedgehog compound,

9 A method according to claim 1, wherein the compound is a first compound derived from a first gene product and is capable of acting synergistically with a second compound that is derived from a second gene product expressed in the extraembryonic tissue, so as to enhance the stimulation of at. . .

10 A method according to claim 9, wherein the second compound is a functional equivalent of a TGF-P family member.

.
further comprising the step of maintaining the cell population in vitro in a culture medium such that step (b) includes providing the compound in the culture medium.

.
to claim 14, wherein the cells are precursor cells from an adult human capable of vascular growth when stimulated by the compound.

25 A method according to claim 24, further comprising causing the compound to access the stem cells, by administering an effective dose of the compound to the animal by

any of oral, intradermal, subcutaneous, transmucosal, intramuscular or intravenous routes.

26 A method according to claim 2, wherein the compound is functionally equivalent to a protein from the bone marrow morphogenic protein (BMP) family.

of treating developmental errors in vascular growth or hematopoiesis in an embryo in utero, comprising:
(a) selecting an effective dose of a compound that is functionally equivalent to a gene product expressed in an extraembryonic tissue; and
(b) causing the compound to access a population of embryonic cells in vivo, so as to stimulate the cells to undergo at least one of.

28 A method according to claim 27, wherein the compound is an agonist of a hedgehog protein-receptor.

29 A method according to claim 27, wherein the compound is a hedgehog protein.

30 A method according to claim 27, wherein the compound is a first compound capable of acting synergistically with a second compound that is derived from a second gene product expressed in the extraembryonic tissue, so as to enhance the stimulation of hematopoiesis in.

A method of treating a subject suffering from an abnormal number of erythroid cells, comprising:
(a) selecting an effective dose of a compound that is functionally equivalent to a gene product expressed in an extraembryonic tissue; and
(b) causing the compound to access a population of hematopoietic stem cells over an effective time so as to modulate the number of cells undergoing.

32 A method according to claim 31, wherein the compound is an agonist of a hedgehog protein-receptor and the hematopoietic stem cells are stimulated to undergo one of proliferation or hematopoiesis.

33 A method according to claim 32, wherein the compound is a hedgehog protein.

34 A method according to claim 31, wherein the compound is a first compound capable of acting synergistically with a second compound that is derived from a second gene product expressed in the extraembryonic tissue, so as to enhance the stimulation of hematopoiesis in.

35 A method according to claim 31, wherein the compound is an antagonist of a hedgehog protein and the hematopoietic stem cells are inhibited from undergoing one of proliferation or hematopoiesis.

38 A method of treating a subject suffering from an ischemia in tissues,
comprising:
(a) selecting an effective dose of a compound that is functionally equivalent to a gene product expressed in an extraembryonic tissue; and
(b) administering the compound to the ischemic site over an effective time so as to stimulate vascular growth within the ischemic tissues.

39 A method according to claim 37, wherein the ischemia is myocardial ischemia.

40 A method according to claim 38, wherein the compound is an agonist of a hedgehog protein-receptor.

41 A method according to claim 40, wherein the compound is a hedgehog protein.

42 A method according to claim 39, wherein the compound is a first compound that is capable of acting synergistically with a second compound that is derived from a second gene product expressed in the extraembryonic tissue, so as to enhance the stimulation of vascular growth.

43 A method of treating abnormally enhanced vascular growth in a subject,
comprising:
(a) selecting an effective dose of a hedgehog compound capable of inhibiting the activity of a gene product expressed in an extraembryonic tissue; and
(b) administering the compound to the subject over an effective time so as to inhibit abnormally enhanced vascular growth.

44 An in vitro assay for determining the activity of a compound capable of modulating hematopoiesis or vascular growth, comprising:
(a) selecting a population of cells from a tissue derived from a fertilized egg of. . .

52 An assay for determining the activity of a compound capable of modulating
15 hematopoiesis or vascular growth, comprising:
(a) selecting a first transgenic animal carrying a marker:c-globin hybrid gene; wherein the . . . animal that is similarly transgenic;
(c) isolating an embryo from the mating during the gestation period; and
(d) determining the effect of the compound on the stimulation of hematopoiesis and vascular growth in the isolated embryo by measuring marker expression.

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